Evaluation of CHROMagar Salmonella Medium for the Isolation of *Salmonella* from Animal Manure

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KEY WORDS: Salmonella, solid media, BGA, XLD, CAS, CHROMagar, chromogenic medium.

ABSTRACT

Salmonella is one of the most important foodborne pathogens that can be transmitted through the consumption of contaminated meat and meat products. Early detection of *Salmonella* in food producing animals and their environment is important for food safety. Three selective media—brilliant green agar (BGA), xylose lysine desoxycholate (XLD) agar, and Salmonella-Shigella agar (SSA)—are commonly used in diagnostic laboratories for the isolation of *Salmonella*, often after enrichment of the samples in a broth before plating on the solid medium. Recently, a new medium called CHROMagar Salmonella (CAS) has become available for the rapid detection of *Salmonella* from human fecal samples. In a preliminary study, we compared this new medium with BGA, XLD, and SSA for the isolation of *Salmonella* from 54 samples of animal manure with and without enrichment in tetrathionate broth for 24 hours. CAS performed poorly as compared with the other 3 media when samples were plated without enrichment. However, the sensitivity of CAS was found to be comparable to the other 3 media after enrichment of samples.

INTRODUCTION

Salmonella causes gastroenteritis in humans and has been ranked as one of the most important foodborne pathogens in the United States.[1] Animal products such as beef, pork, poultry, eggs, raw milk, and milk products can act as vectors of *Salmonella*. Early detection in food producing animals and their environment can help reduce fecal shedders of *Salmonella* from animal production systems, thus breaking the cycles of infection and transmission.

Several techniques, such as polymerase chain reaction (PCR),[2] immunological techniques,[3] and automated identification systems,[4] are available for the detection of *Salmonella* in a variety of samples. These techniques are not routinely used in all diagnostic laboratories because of high capital expenditure and the need for highly skilled technicians to perform these tests. Thus, plat-

Table 1. Sensitivity of Various Media for Isolation of *Salmonella* from Animal Manure with and Without Enrichment in Tetrathionate Broth*

	Number of positive samples (Number of *Salmonella*)							
	Without enrichment				With enrichment			
Animal species	BGA	SSA	XLD	CAS	BGA	SSA	XLD	CAS
Cattle	2 (7)	0	1 (1)	0	0	1 (2)	0	0
Turkey	0	0	0	0	0	0	0	0
Swine	1 (2)	0	1 (1)	1 (1)	0	0	0	0
Canine	1 (3)	1 (3)	2 (5)	1 (2)	0	0	0	0
Total	2 (7)	1 (2)	1(1)	0	2 (5)	1 (3)	3 (6)	2 (3)

*BGA indicates brilliant green agar; SSA, Salmonella-Shigella agar; XLD, xylose lysine desoxycholate agar; and CAS, CHROMagar Salmonella.

ing on solid media after enrichment remains the method of choice for most laboratories around the globe. The solid media most commonly used are brilliant green agar (BGA), xylose lysine desoxycholate (XLD) agar, and Salmonella-Shigella agar (SSA). However, not all media are universally sensitive for the detection of *Salmonella* from all sample types[5,6] and some do not support the growth of certain serovars of *Salmonella*.[5,7,8] Recently, a new medium called CHROMagar Salmonella (CAS) has become available for the selective isolation of *Salmonella.*[9,10] This medium is considered to be highly sensitive and specific for the detection of *Salmonella* in human stool samples[9] including certain fastidious serotypes such as *S typhi* and *S paratyphi* A.

Microbial analysis of animal manure has been used as an indicator of the general health of animal herds and can also be used in the identification, and subsequent removal, of fecal shedders from the food chain. A limited number of studies have evaluated the suitability of solid media for the detection of *Salmonella* in animal manure.[11,12] The objective of this preliminary study was to determine the suitability of CAS for the isolation of *Salmonella* from animal manure samples and compare it with 3 other commonly used media: BGA, SSA, and XLD agar.

MATERIALS AND METHODS

Samples

A total of 54 samples, cattle (n = 24), pigs (n = 7), dogs (n = 13), and turkeys (n = 10), were tested in this study. All samples were stored at 4˚C and were tested within 48 hours of collection.

Media, Sample Preparation, and Plating

Plates of BGA, SSA, XLD agar (Becton Dickinson, Sparks, Md), and CAS (CHROMagar Microbiology, Paris, France) were prepared as per manufacturers' instructions. Before pouring plates, CAS was supplemented with 10 μg mL^{-1} of cefulodin sodium (Sigma, St. Louis, Mo) to inhibit the growth of *Pseudomonas*.[9] Samples were plated on all 4 media with and without enrichment. For direct plating, 1 g of sample was suspended in 5 mL of buffered peptone water (BPW) and mixed well; a loopful was plated on each of the 4 solid media. For enrichment, 1 g of sample was suspended in 5 mL of tetrathionate broth (TTB, Remel, Los Angeles, Calif) followed by incubation at 42˚C for 18 to 24 hours. A loopful of enriched culture was then plated on each of the 4 solid media. The inoculated plates were incubated at 37˚C for 24 hours and then examined for the appearance of *Salmonella*-like colonies.

Colony Evaluation and *Salmonella* Identification

Colonies showing characteristic color on respective media (n = 1–4 per plate) were picked and inoculated on slants of triple sugar iron agar (TSI, Becton Dickinson, Sparks, Md) followed by incubation at 37˚C for 24 hours. Lactose non-fermenters with or without H_2S production were selected

and subjected to a set of biochemical tests eg, sugar fermentation (dextrose, sucrose, mannitol, maltose, and ducitol), motility, ornithine, indole production, and C_8-estrase spot test.[13] The latter test was performed using MUCAP reagent (Biolife Italiana S.r.l, Milan, Italy).

Spiking of Manure Samples

Approximately 50–g samples of turkey and swine manure were sterilized by autoclaving. Six hundred microliters of different dilutions (10^{-1} to 10^{-9}) of an overnight culture of *S typhimurium* (initial count = 9.8 x 10^8 colony forming units mL^{-1}), grown in tryptic soy broth (TSB), were added to 3 g of each sterile sample. After thorough mixing with a sterile spatula, a 1-g sample was suspended in 5 mL of BPW, while another 1g of sample was suspended in 5 mL of TTB for enrichment. Samples with and without enrichment were plated on all 4 media (100 µL/dilution/plate). After incubation at 37˚C for 24 hours, the numbers of *Salmonella* colonies were counted.

RESULTS

Evaluation of Colonies on Different Solid Media

Presumptive positive colonies were isolated on the basis of characteristic colony color and morphology on different media. On BGA, *Salmonella* colonies were surrounded by a pink zone, whereas on SSA and XLD agar, the colonies appeared as black centered because of H_2S production. On CAS medium, *Salmonella* colonies were mauve colored while non-*Salmonella* colonies were blue or violet. On XLD and SSA plates, non-*Salmonella* colonies appeared white with yellow background. On BGA plates, non-*Salmonella* colonies were white.

***Salmonella* Detection in Spiked Samples**

S typhimurium spiked in turkey and swine manure could be detected on all 4 media with and without enrichment. However, the range of dilution over which the *Salmonella* colonies were detected varied. After enrichment in TTB, *Salmonella* could be detected on all 4 media at all dilutions of turkey and swine manure (10^{-1} to 10^{-9}; initial count = 9.8 x 10^8 mL^{-1}). When spiked samples of swine manure were plated without enrichment, *Salmonella* colonies developed on all 4 media at 10^{-1} to 10^{-7} dilutions but not at 10^{-8} to 10^{-9} dilutions. The number of colonies at 10^{-7} ranged from 26 to 42 on different media. When spiked turkey manure was plated without enrichment, *Salmonella* colonies were detected on all 4 media at only 10^{-1} to 10^{-5} dilutions. The number of colonies on different media ranged from 20 to 35 at 10^{-5} dilution. The colony number and dilution comparison suggests that all 4 media were a bit more sensitive for *Salmonella* detection in swine manure than in turkey manure.

***Salmonella* Detection in Field Samples**

The results of *Salmonella* isolation from 54 field samples are shown in Table 1. The number of confirmed *Salmonella* colonies on different media varied from 0 to 7 and the number of positive manure samples was between 0 and 3. Without enrichment, none of the samples was positive on CAS media while 1 to 2 samples were found positive for *Salmonella* on the other 3 media. With enrichment, at least one sample was found positive on each of the 4 media, with XLD yielding the maximum numbers of positive samples (n = 3). Of the 3 cattle manure samples found positive, one sample was positive for *Salmonella* after enrichment on BGA, XLD, and CAS, while one sample was positive on BGA and XLD without enrichment. Of the two swine manure samples found positive after enrichment, one was found positive on all 4 media. No *Salmonella* colony was detected in any of the canine samples, whereas one turkey manure sample yielded *Salmonella* on SSA.

DISCUSSION

Salmonella is one of the most important foodborne pathogens and infects millions of humans annually. Hence, monitoring the general health condition of food-producing animals using rapid methods for the detection of *Salmonella* is extremely important.

In this preliminary study, we evaluated 4 commercially available media for their suitability in detection of *Salmonella* (with and without pre-enrichment in tetrathionate broth) from 54 samples of animal manure. Without enrichment, BGA was found to be the most sensitive method, yielding a total of 7 confirmed *Salmonella* isolates from 2 samples. In contrast, none of the samples yielded *Salmonella* by direct plating on CAS. These results are in contrast to those reported by Maddocks et al[10] in which direct plating on CAS was reported to be 100% sensitive for the detection of *Salmonella* from human fecal samples. However, our results are in agreement with those of Barkocy-Gallagher et al[14] in which they reported excellent *Salmonella* recovery from a variety of bovine samples after direct plating on BGA supplemented with sulfadiazine but not with CAS and Rambach agar.

Following enrichment in TTB, the maximum number of samples were found positive on XLD agar (n = 3). This is in agreement with Maddocks et al,[10] who reported 100% sensitivity of XLD for detection of *Salmonella* after enrichment from human stool samples. Similarly, Cooke et al[15] reported high sensitivity and specificity of XLD when *Salmonella* test strains were used for media evaluation. The suitability of XLD for the isolation of *Salmonella* from human feces and municipal waste water has also been reported.[6,16]

The results obtained with the field samples were different than those obtained with experimentally spiked samples of turkey and swine manure. This could have been due to the absence of background microflora since manure samples used in the experimental study were sterilized before spiking. All 4 media showed greater sensitivity in detecting *Salmonella* in spiked samples than in field samples probably because of the high inoculum (9.8×10^8 CFU mL^{-1}) used in spiked samples. All 4 media were equally sensitive in detecting *Salmonella* from spiked samples except that all 4 media were almost 100-fold more sensitive for the detection of *S typhimurium* in swine manure than in turkey manure. This may be due to the semi-solid nature of swine manure, making it easier to mix the bacterial inoculum with the sample. The solid nature of turkey manure makes it difficult to properly mix the inoculum with the manure. In addition, turkey manure may contain inhibitors that might interfere with the growth of *Salmonella*. Further studies are needed to explore this phenomenon.

In the present study, lower sensitivity of CAS for field samples could be attributed in part to difficulty in distinguishing between mauve and violet colored colonies.[10] Since a small number of samples were tested in the present study, it is difficult to conclude whether the CAS medium is as sensitive for the detection of *Salmonella* in animal manure samples as in human stools.[10] Further studies are needed to evaluate the use of CAS medium as a screening medium.

ACKNOWLEDGMENTS

We thank Karen Olsen for technical assistance. This study was supported in part by a USDA-North Central Region SARE grant, No. UNE/25-6205-0034-023/USDA SARE.

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